Excretory mechanisms for cationic drugs

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Summary

In this thesis some molecular aspects of transmembrane transport of drugs are studied, as mediated by P-glycoprotein and other related transport proteins. These membrane proteins are present in liver, kidneys and intestine and it is hypothesized that they are involved in the elimination of cationic drugs from the body via bile, urine and small intestinal contents (faeces).

Summary

The literature that describes the mechanisms of organic cation transport in the liver is summarized in the introductory *chapter, 2*. The current knowledge about carrier-mediated hepatic uptake processes of cationic compounds as well as the studies on the identification of various secretory processes involved in the biliary secretion of organic cations is reviewed. For example, the molecular identification and cloning of a member of these transport proteins is described.
In *chapter 3* the potential influence of Protein Kinase C (PKC)-mediated phosphorylation of transport proteins on organic cation secretion into bile is described. It was found that activating agents such as phorbol 12-myristate 13-acetate (PMA) and vasopressin can largely stimulate the biliary excretion rate of TBuMA, whereas the PKC antagonist staurosporin inhibited the TBuMA biliary excretion rate. cAMP-mediated phosphorylation did not seem to play a significant role in the biliary excretion of organic cations. It is speculated that an ATP-dependent transport protein, localized at the hepatocyte canalicular plasma membrane domain, is involved in the cationic drug secretion into bile. Apart from P-glycoprotein (P-gp) various other ATP-dependent transporters are potential candidates.
The molecular mechanism of cationic drug transport across the canalicular membrane was further studied in canalicular membrane vesicle enriched liver plasma membrane preparation (cLPM) (*chapter 4*). This chapter describes the potential role of a organic cation: proton exchanger that can mediate the transport of TBuMA in cLPM vesicles. The observed transport was depended on an outwardly directed pH-gradient and could be cis-inhibited by several cationic model drugs such as decynium22 and vecuronium. Addition of ATP did not stimulate TBuMA uptake into cLPM vesicles. [^{3}H]-TBuMA uptake into cLPM vesicles was trans-stimulated upon preloading vesicles with non-radioactive labeled TBuMA. This indicated that this organic cation antiporter differs from the primary active P-glycoprotein and can function bidirectionally.
Organic cation transport in the intact rat liver was extensively investigated and described in *chapter 5*. This chapter deals with the potential interaction during membrane transport at the bile canalicular level during co-administration of cationic drugs. A significant correlation between the size reduction of biliary output of the investigated model compounds doxorubicin, rocuronium and TBuMA, and the lipophilicity of a series of lipophilic P-gp substrates was found. This indicates that the relative affinity of the cationic drug for the supposed transport protein, is influenced by the hydrophobic interaction with the particular transporters. Alternatively, this physicochemical feature may influence the partitioning of cationic drugs into the lipid-phase of the membrane, a process that may be involved in the association of substrates to drug-binding site(s) in the potential transport protein.

Since an excess of the competing agents did only produce a partial inhibition of cationic drug secretion into bile and since transport inhibition was not mutual in some cases, we postulated that besides P-glycoprotein other carriers are probably involved in drug secretion. One example could be the organic cation: proton antiporter described above. Alternatively, major differences in the relative affinity

and/ or accessibility to the transporters of the model drugs and the various inhibitors used could play a role.

A mdr1a P-gp deficient mouse model was generated to further substantiate the contribution of drug transporting P-gp to elimination of iv administered cationic drugs from the body (see *chapter 6*). Absence of the mdr1a P-gp resulted in a significant reduction of the biliary and intestinal clearance of organic cations compared to the wild-type. This implies that P-gp is likely to be involved both in the hepatobiliary as well as in the intestinal secretion of cationic compounds like TBuMA, APM and vecuronium in mice. Urinary clearance of small type1 cationic compounds seemed also to be affected by the absence of the mdr1a P-gp, although for TBuMA the difference between wild-type and mdr1a (-/-) mice did not reach statistical significance (p=0.09). We hypothesized that the residual biliary output of cationic compounds in mdr1a P-gp deficient mice is mediated by mdr1b P-gp and/or the functionally described organic cation: proton antiporter that was earlier mentioned.

The role of mdr1-type (drug transporting) P-gp in cationic drug elimination was further investigated *in vivo* using the *mdr1a/ mdr1b* gene "knockout" mouse model (*chapter 7*) that lacks both mdr1a as well as the mdr1b P-gp isoform. These studies provided further evidence for an important role of mdr1-type P-gp in hepatic as well as intestinal secretion of organic cations: the excretion of the cationic model compounds TBuMA, APM and vecuronium were at least 70% reduced in absence of the mdr1a/1b P-gps. Interestingly the renal clearance of both the type1 and the type2 cations was significantly increased in absence of both mdr1a and mdr1b P-gp. Vecuronium renal clearance was even about 5-fold increased. Interestingly (*chapter 7*) in mdr1a/1b(-/-) mice the clearance of TBuMA as well as that of APM shifted to the renal secretory route that resulted in an increased renal clearance that compensated for the reduced hepatic and intestinal clearance. The mechanism causing the increased renal clearance of type1 cationic compounds in the mdr1a/1b P-gp deficient mice remains to be clarified. Complete absence of drug transporting (mdr1-type) P-gp could result in secondary changes that influence organic cation elimination from the body.

Therefore, we more definitely established the direct involvement of mdr1-type P-gp in organic cation transport by using epithelial cells that were transfected with various cDNA's encoding mdr1-type P-gps (*chapter 8*). Apical directed transport of all tested compounds in polarized grown epithelial LLC-PK1 cells was significantly increased when P-gp was expressed. This indicates that P-gp can mediate the transport of aliphatic as well as of more bulky cationic compounds in such a cell system. These observations also support the *in vivo* data indicating that mdr1-type P-gps are involved in the elimination form the body of a wide variety of amphiphilic drugs.